Hardware Guide

API 5000™ System

D1000092206 B
May 2010

AB SCIEX

AB SCIEX
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AB SCIEX LP is ISO 9001 registered.

Printed in Canada.

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System Overview

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The API 5000™ LC/MS/MS system includes a triple quadrupole mass spectrometer, a Turbo V™ ion source, a computer, and the Analyst® software.

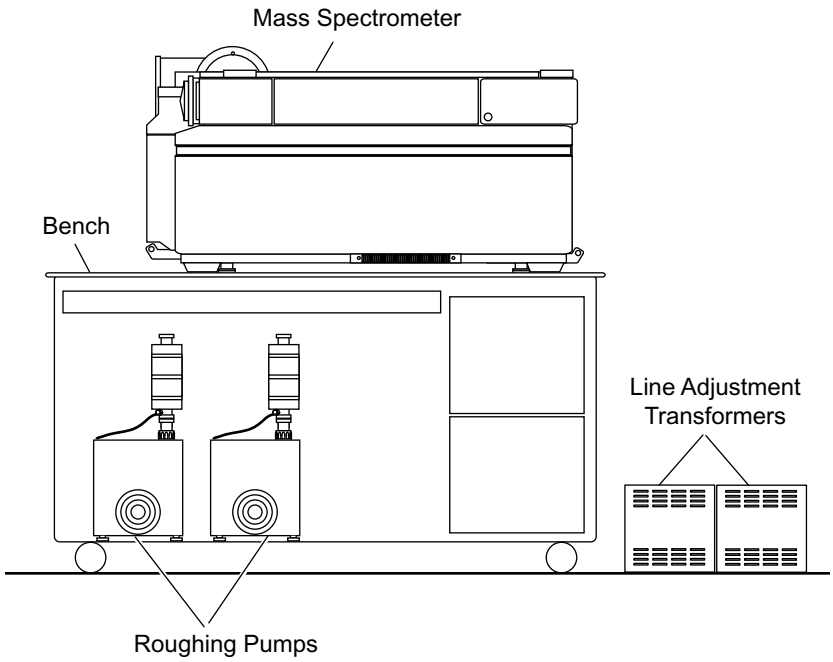

Figure 1-1 API 5000™ system and bench

WARNING! If you need to move the system, contact an FSE to assist you. Risk of personal injury or instrument damage

Note: Before you operate the instrument, make sure you have read the Safety Practices guide.

Principles of the System

This section describes features and applications of the API 5000™ system.

Applications for the System

The API 5000 system is designed for quantitative analysis of small molecules.

This application involves measurement of specific molecular weight compounds, usually a drug or metabolite in urine or other body fluid, and their resulting

fragment ions for determining the exact quantity of the compound in the sample of interest. Quantitation is performed using a standard curve of mass spectrometer signal intensity for various known concentrations of the compound. The signal in the test sample is compared to the standard curve to determine the concentration.

The typical scan type used for this application in the API 5000 system, as well as all triple quadrupole instruments, is MRM (Multiple Reaction Monitoring).

Features of the Instrument

The API 5000 LC/MS/MS system combines all of the features of the API 4000™ LC/MS/MS system with the additional benefits of improved sensitivity and enhanced signal-to-noise ratio. It allows comprehensive analysis of biopharmaceutical compounds and it has the specificity needed for new drug development. It has the following features:

- A wide diameter intake orifice which improves sensitivity by flowing more ions into the vacuum chamber.
- A QJet® ion guide that prefocuses the ions before they enter the vacuum chamber for higher sensitivity and signal-to-noise ratio.
- Improved Q2 lenses reduce CAD gas leakage into the vacuum chamber and improve sensitivity and signal-to-noise ratio.
- Higher quadrupole operating frequency, which improves ion transmission and increases sensitivity, especially for low ion masses.
- Higher sensitivity in all RF/DC scan modes.
- Scanning in a mass range of m/z 5–1250 in quadrupole mode.
- Enhanced resolution at reduced scan speeds.

For pharmaceutical and pharmacokinetic samples, MS/MS has the sensitivity and specificity needed to analyze hundreds of samples per day without requiring extensive sample preparation.

How the Instrument Works

Mass spectrometry measures the mass-to-charge ratio of ions to identify unknown compounds, to quantify known compounds, and to provide information about the structural and chemical properties of molecules. The API 5000 system has a series of quadrupole filters that transmit ions according to their m/z (mass-to-charge ratios). The first quadrupole in this series is the QJet ion guide located between the orifice plate and Q0. The QJet ion guide does not filter ions, but focuses them before they enter Q0. By prefocusing the larger ion flux created by the wider orifice, the QJet ion guide increases instrument sensitivity and improves the signal-to-noise ratio. In Q0 the ions are again focused before passing into Q1.

Q1 is a filtering quadrupole that sorts the ions before they enter Q2. Q2 is a collision cell in which ions can be broken into fragments by collisions with gas molecules. This technique allows users to design experiments that measure the m/z of product ions to determine the composition of the parent ions. After passing through Q2 the ions enter Q3 for additional filtering, and then enter the detector. In the detector, the ions create a current that is converted into a voltage pulse. The voltage pulses leaving the detector are directly proportional to the quantity of ions entering the detector. The instrument monitors these voltage

pulses and converts the information into a signal. The signal represents the ion intensity for a particular m/z and the instrument displays this formation as a mass spectrum. For more information, see Figure 1-2 *Ion optics path*.

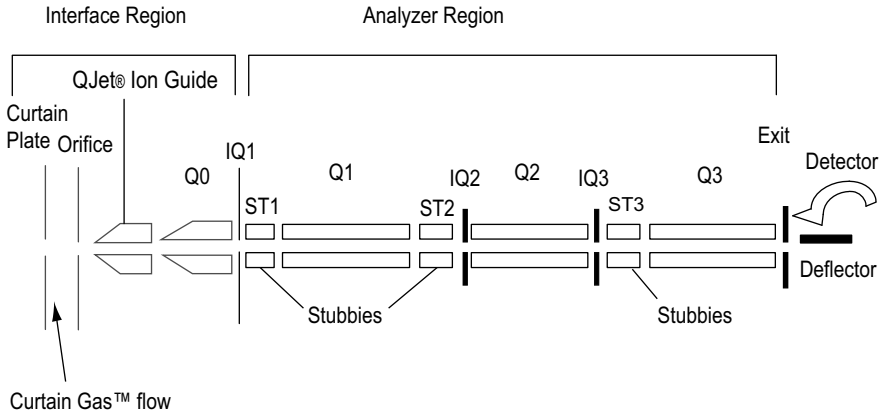

Figure 1-2 Ion optics path

The API 5000 system uses the Turbo V™ ion source to produce ions from liquid samples. The Turbo V source can use either the TIS (TurboIonSpray®) probe or the APCI (atmospheric pressure chemical ionization) probe. The instrument is configured to perform complex MS/MS analysis, but for less rigorous analytical requirements it can perform single MS scans.

Parts of the Instrument

This section contains diagrams of the instrument and general descriptions of its components.

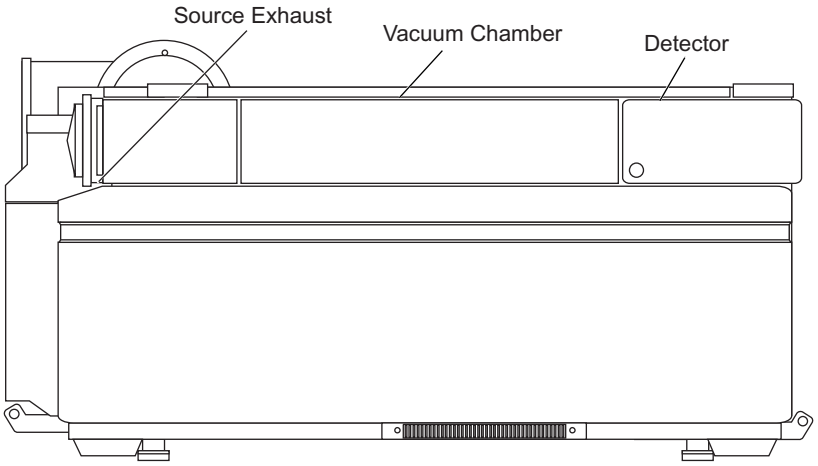

Figure 1-3 Front view

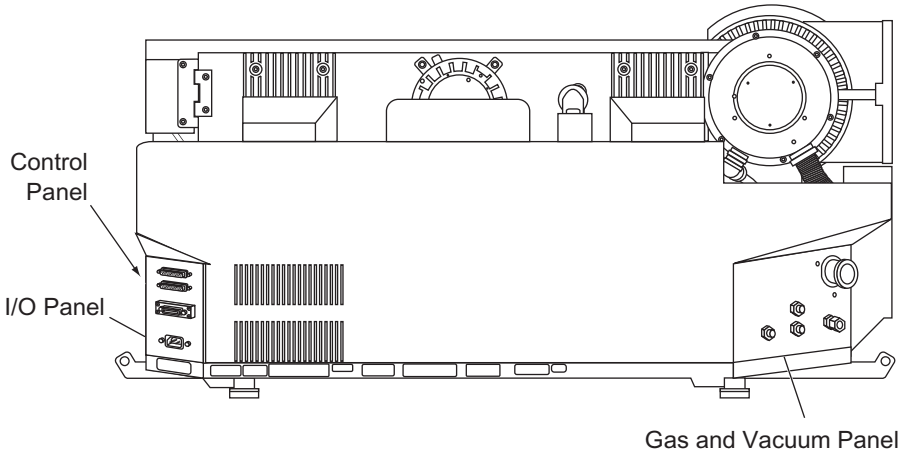

Figure 1-4 Back view

I/O Panel

The input/output panel on the right-hand side of the chassis has a socket for the external AC power supply, the IEEE-488 (GPIB) connections to the computer, the AUX I/O to the peripherals, and a sources connector for optional sources.

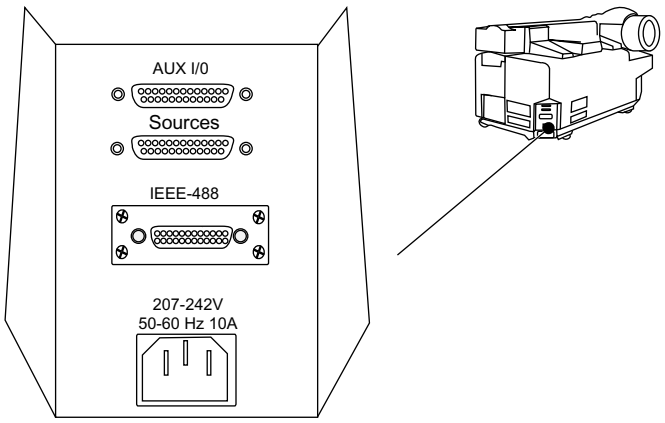

Figure 1-5 I/O panel

Gas and Vacuum Panel

The vacuum line to the external roughing pumps is connected through the gas and vacuum panel through the port labeled backing pump. The panel also houses the gas supply connections and the external connection for the source exhaust pump.

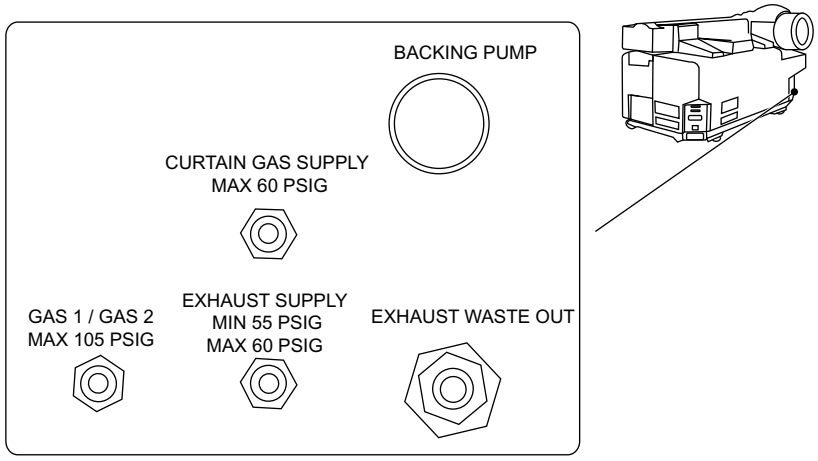

Figure 1-6 Gas and vacuum panel

Control Panel

The control panel is located on the right side of the instrument. It encloses the power distribution module.

The control panel contains the main power switch, four fuses, and two Indicator/Reset buttons. The Vacuum Status button (green) and the Fault button (red) indicate the vacuum status on the system controller board. When triggered together, simultaneously pushed and released, the buttons reset the two microprocessors on the system controller. This has an effect similar to restarting the computer.

CAUTION! Resetting the instrument using these buttons interrupts any scans and results in the loss of any unsaved files and data.

When the operational vacuum conditions are satisfied and the instrument is in analysis mode, the Vacuum Status button is illuminated and the Fault button is extinguished. The Fault button flashes when a vacuum fault is detected. In Pump-Down mode, the buttons indicate the status of the Pump-Down sequence.

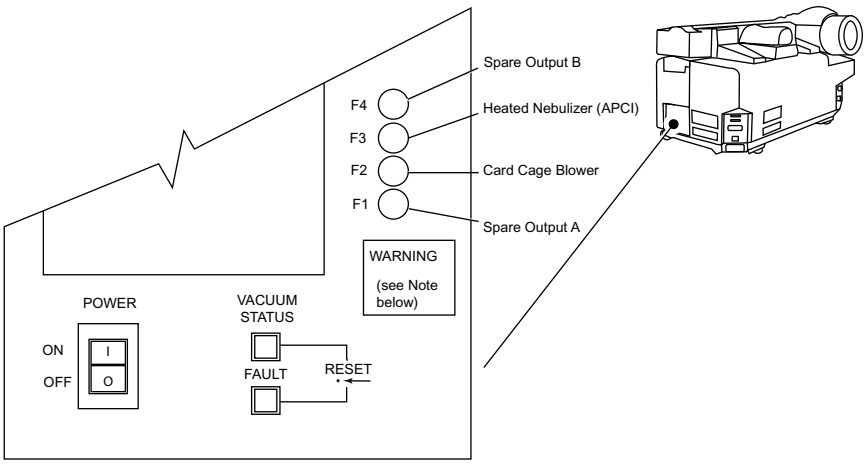

Figure 1-7 Control panel

Source Exhaust System

The source exhaust system is a safety feature that isolates the sample vapors and exhaust products from the laboratory environment. The source requires that the source exhaust system is properly connected and functioning. The exhaust pump draws the solvent vapors from the enclosed source housing and delivers them to a trap at the rear of the instrument chassis. It is recommended that these vapors be passed through this trap and then vented to a fume hood or outside port.

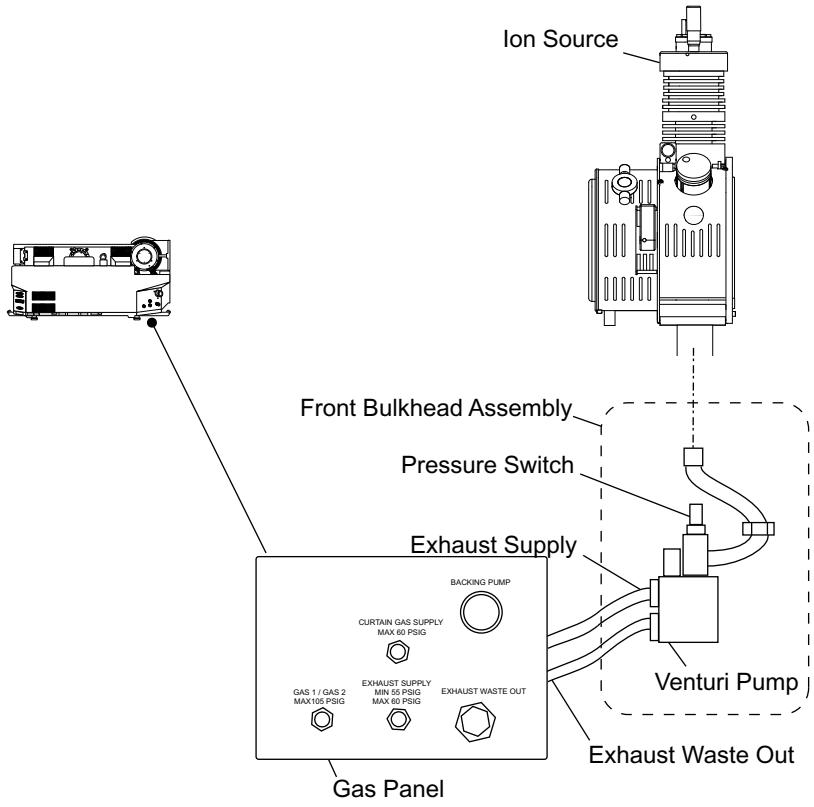

Figure 1-8 Source exhaust system

WARNING! Hazardous Materials: Take all necessary precautions to ensure the safe disposal of the source exhaust gas. Follow all applicable local regulations.

A filtered nitrogen or zero air gas supply (oil free) is delivered to the source exhaust pump at pressures indicated on the gas panel at the back of the instrument. The previous figure shows the exhaust supply connection points for the API 4000™ and API 5000™ systems.

Vacuum Chamber

The vacuum chamber houses the mass filter rail, including the ion optics, the quadrupole rod sets, the collision cell, and the ion detector.

Shutting Down and Turning on the System

Use the following procedures if you need to shut down or turn on the system.

To shut down the system

1. In the Analyst® software, complete or stop any ongoing scans.
2. Turn off the sample flow to the instrument.

CAUTION! Potential Instrument Damage: Shut off the sample flow before you shut down the instrument.

3. In the Analyst software, deactivate the hardware profile and then close the application software.
4. Stop the Analyst® Service. (See the *Software Reference Guide* for the Analyst software.)

CAUTION! Leave the roughing pumps running for a minimum of 15 minutes after turning off the instrument's main power switch. When the main power switch is turned off, the turbo pumps continue to rotate without power for a few minutes and continue to provide vacuum to the vacuum chamber. If, during this time, the roughing pumps are turned off, the pressure in the vacuum line between the roughing pumps and the turbo pumps increases. The increase in back pressure can create an incorrect load on the turbo pumps' bearings and can cause a catastrophic failure of the turbo pumps.

CAUTION! If the instrument is to be shut down for any length of time, we recommend that the vacuum chamber be vented to prevent exhaust from the roughing pump being sucked back into the vacuum chamber. To vent the vacuum chamber, follow steps 5 to 7.

CAUTION! If the vacuum chamber is not going to be vented while the instrument is shut down, we recommend the roughing pump remain turned on to prevent the pump exhaust from being sucked back into the vacuum chamber. If you do not want to vent the vacuum chamber, skip steps 6 and 7.

5. Turn off the main power to the instrument. As you face the instrument where the instrument name is visible and with the source to your left, the switch is located on the bulkhead at the back right corner of the chassis.
6. After fifteen minutes, turn off the roughing pumps. The power switch is located beside the power supply input attachment on the roughing pump.

Note: The roughing pump has its own power toggle switch and must be turned off manually. The roughing pump is not controlled remotely by the system controller.

7. Let the vacuum chamber vent naturally through the orifice for ten minutes to allow the instrument to reach atmospheric pressure.
8. Unplug the mains power cord to the instrument from the rear bulkhead near the main power switch for the instrument.

To turn on the system

Certain conditions outside the direct control of the instrument firmware must be satisfied before the turbo pumps will be initiated. The Curtain Gas™ supply must be turned on at the source, and the roughing pump must be turned on manually.

Interlocks (pressure switches) monitored by the firmware detect if the Curtain Gas supply and the roughing pump are switched on. If the interlocks are not satisfied, the turbo pumps are not initiated.

CAUTION! If the ion source is removed, the system electronics will be disabled, interrupting any data acquisition tasks. The turbo pump and the vacuum system will not be affected.

1. Turn on the roughing pump, if it was turned off. The power switch is located beside the power supply input attachment on the roughing pump.

Note: The roughing pump has its own power toggle switch and must be turned on manually. The roughing pump is not controlled remotely by the system controller.

2. Make sure that all gas supplies are flowing correctly to the instrument.
3. Plug the mains power cord into the bulkhead.
4. Turn on the main power switch.
5. Make sure that the GPIB (general purpose interface bus) cable is connected to both the instrument and the computer.
6. Turn on the computer, if it was turned off, and then start the Analyst® software.

Source/Gas Parameters

The parameters in Table 1-1 are optimized for different LC conditions involving flow rate. For more information about the parameters, see the Analyst® software Help system.

Note: We recommend that you run the instrument with the Curtain Gas™ flow set to at least 20 to maintain good instrument performance.

Table 1-1 Source/Gas Parameters

Parameter	Value
Curtain Gas (CUR)	20
IonSpray Voltage (IS)	5000
Temperature (TEM)	700
Ion Source Gas (GS1)	60
Ion Source Gas 2 (GS2)	60

Turbo V™ Ion Source Settings

Table 1-2 shows the recommended starting values for the X- and Y-axis parameters. For more information, see the *Turbo V™ Ion Source Operator's Guide*.

Table 1-2 Vertical and Horizontal Parameters

Parameter	Value
X-axis	5
Y-axis	5

Compound Parameters

In general, you can use the preset values for most of the parameters in Table 1-3. For more information about the parameters, see the Analyst® software Help system.

Table 1-3 Compound Parameters

Parameter	Value
CAD Gas	Use the preset value and optimize for your compound.
DP (Declustering Potential)	Use the preset value and optimize for your compound.
EP (Entrance Potential)	Use the preset value.
CXP (Collision Cell Exit Potential)	Use the preset value and optimize for your compound.
CE (Collision Energy)	Use the preset value and optimize for your compound.
IE1 (Ion Energy 1) — *For experienced instrument operators only*	Do not adjust.
IE3 (Ion Energy 3) — *For experienced instrument operators only*	Do not adjust.

Related Documentation

The guides and tutorials for the instrument and the Analyst software are installed automatically with the software and are available from the Start menu: All Programs > AB SCIEX. A complete list of the available documentation can be found in the online Help. To view the Analyst software Help, press F1.

Technical Support

AB SCIEX and its representatives maintain a staff of fully-trained service and technical specialists located throughout the world. They can answer questions about the instrument or any technical issues that may arise. For more information, visit the web site at http://www.absciex.com.

API 5000™ Instrument Parameters

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The following table contains generic parameters for the API 5000™ instrument. The first number under each scan type is the preset value; the range of numbers is the accessible range for each parameter.

Table 2-1 API 5000™ Instrument Parameters

Parameter ID	Access ID	Positive ion mode			Negative ion mode		
		Q1	Q3	MS/MS	Q1	Q3	MS/MS
CUR	CUR	10 10 to 50	10 10 to 50	10 10 to 50	10 10 to 50	10 10 to 50	10 10 to 50
CAD	CAD	0 n/a	1 0 to 12	4 0 to 10	0 n/a	1 0 to 12	4 0 to 12
IS(1)(2)	IS(1)(2)	5500 0 to 5500	5500 0 to 5500	5500 0 to 5500	–4500 –4500 to 0	–4500 –4500 to 0	–4500 –4500 to 0
NC(3)	NC(3)	3 0 to 5	3 0 to 5	3 0 to 5	–3 –5 to 0	–3 –5 to 0	–3 –5 to 0
TEM(2)(3)	TEM(2)(3)	0 0 to 750	0 0 to 750	0 0 to 750	0 0 to 750	0 0 to 750	0 0 to 750
OR (DP = OR)	DP	120 0 to 400	120 0 to 400	120 0 to 400	–100 –400 to 0	–100 –400 to 0	–100 –400 to 0
Q0 (EP = –Q0)	EP	10 15 to 2	10 15 to 2	10 15 to 2	–10 –15 to –2	–10 –15 to –2	–10 –15 to –2
IQ1 (IQ1 = Q0 + offset)	IQ1	Q0 + (–1) –0.5 to –2	Q0 + (–1) –0.5 to –2	Q0 + (–1) –0.5 to –2	Q0 + 1 0.5 to 2	Q0 + 1 0.5 to 2	Q0+1 0.5 to 2
ST (ST = Q0 + offset)	ST	Q0 + (–7) –8 to –4	Q0 + (–7) –8 to –4	Q0 + (–7) –8 to –4	Q0 + 7 4 to 8	Q0 + 7 4 to 8	Q0 + 7 4 to 8
RO1 (IE1 = Q0 – RO1)	IE1	1 0.5 to 2	n/a	1 0.5 to 2	–1 –2 to –0.5	n/a	–1 –2 to –0.5
RO1 (IE1 = Q0 + offset)	RO1	n/a	Q0 + (–2) –0.5 to –2	n/a	n/a	Q0 + 2 0.5 to 2	n/a
(1)IonSpray™ ion source (2)TurboIonSpray® ion source (3)Heated Nebulizer (4) 1=ON and 0=OFF (5)DuoSpray™ ion source, 1=TIS, and 2=HN							

Table 2-1 API 5000™ Instrument Parameters (cont'd)

Parameter ID	Access ID	Positive ion mode			Negative ion mode		
		Q1	Q3	MS/MS	Q1	Q3	MS/MS
IQ2 (IQ2 = Q0 + offset)	IQ2	Q0+ (–20) –100 to –8	Q0+ (–20) n/a	Q0+ (–20) n/a	Q0 + 20 100 to 8	Q0 + 20 n/a	Q0 + 20 n/a
RO2	RO2	–100 –200 to 200	–20 –145 to –2	n/a	100 –200 to 200	20 2 to 145	n/a
RO2 (CE = Q0 – RO2)	CE	n/a	n/a	30 5 to 130	n/a	n/a	–30 –130 to –5
ST3	ST3	–120 –200 to 200	n/a	n/a	150 –200 to 200	n/a	n/a
ST3 (CXP = RO2 – ST3)	CXP	n/a	20 0 to 55	15 0 to 55	n/a	–20 –55 to 0	–15 –55 to 0
RO3	RO3	–150 –200 to 200	n/a	n/a	100 –200 to 200	n/a	n/a
RO3 (IE3 = RO2 – RO3)	IE3	n/a	2 –0.5 to 5	2 –0.5 to 5	n/a	–1.5 –5 to 0	–1.5 –5 to 0
DF	DF	–200 –400 to 0	–200 –400 to 0	–200 –400 to 0	200 0 to 400	200 0 to 400	200 0 to 400
CEM	CEM	2000 500 to 3297	2000 500 to 3297	2000 500 to 3297	2000 500 to 3297	2000 500 to 3297	2000 500 to 3297
GS1	GS1	20 0 to 90	20 0 to 90	20 0 to 90	15 0 to 90	15 0 to 90	20 0 to 90
GS2	GS2	0 0 to 90	0 0 to 90	0 0 to 90	0 0 to 90	0 0 to 90	0 0 to 90
ihe[4]	ihe	1 0 or 1	1 0 or 1	1 0 or 1	1 0 or 1	1 0 or 1	1 0 or 1
IHT	IHT	40 10 to 250	40 10 to 250	40 10 to 250	40 10 to 250	40 10 to 250	40 10 to 250
svp[5]	svp	1 1 or 2	1 1 or 2	1 1 or 2	1 1 or 2	1 1 or 2	1 1 or 2

(1)IonSpray™ ion source (2)TurboIonSpray® ion source (3)Heated Nebulizer (4) 1=ON and 0=OFF (5)DuoSpray™ ion source, 1=TIS, and 2=HN